

NEWS AND VIEWS

PERSPECTIVE

Hybrid zones and the speciation continuum in *Heliconius* butterflies

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Tropical butterflies in the genus *Heliconius* have long been models in the study of the stages of speciation. *Heliconius* are unpalatable to predators, and many species are notable for multiple geographic populations with striking warning colour pattern differences associated with Müllerian mimicry. A speciation continuum is evident in *Heliconius* hybrid zones. Examples range from hybrid zones across which (a) there is little genetic differentiation other than at mimicry loci, but where hybrids are common, (b) to 'bimodal' hybrid zones with strong genetic divergence and few hybrids, (c) through to 'good' sympatric species, with hybridization extremely rare or absent. Now, in this issue of *Molecular Ecology*, Arias *et al.* (2012) have found an intermediate case in Colombian *Heliconius cydno* showing evidence for assortative mating and molecular differences, but where hybrids are abundant.

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Hybrid zones in neotropical *Heliconius* butterflies have long fascinated evolutionary biologists and informed about stages of speciation. Henry Walter Bates was the first to study *Heliconius* on a scientific basis, and he was also the first to explain mimicry among unrelated species via natural selection. Together with Fritz Müller, Bates was able to explain the selective advantage of this mimicry between edible and unpalatable species (Batesian mimicry) and among unpalatable species (Müllerian mimicry): birds, having learnt to avoid the warning colours of one inedible species, thereafter shun other species with convergent or 'mimetic' colour patterns (Bates 1862; Müller 1879).

It is less well known that Bates, who was particularly interested in the origin of species after reading Darwin's

works, was also the first to document a hybrid zone and assess its implications for understanding the continuum of speciation (Bates 1863). He carried out this work on a hybrid zone between colour pattern races of *Heliconius melpomene* in the Brazilian Amazon. Recent work with heliconiines has shown how mimicry and speciation are closely intertwined across the adaptive radiation in this group (Linares 1997; McMillan *et al.* 1997; Jiggins *et al.* 2001; Arias *et al.* 2008). Now, a new paper closes the gap between species and geographic races still further (Arias *et al.* 2012).

Speciation appears to be more of a problem for humans to understand than it is for the organisms that do it. Learned books tell us that speciation was a conundrum not solved by Darwin, and one that is not clearly understood even today. Nonetheless, many intermediate stages of ongoing speciation are evident both below the species level (ecotypes, ecological races and host races) and above the species level (involving hybridization and gene flow between species and hybrid speciation). Speciation clearly exists as a continuum across the species boundary, and assessing these intermediate stages is a key to understanding the process.

Studies involving the genus *Heliconius* have been major recent contributors to our knowledge of the speciation continuum. An early study used hybrid zone dynamics to measure the strong natural selection on mimicry and showed how this selection could stabilize narrow zones of hybridization between Peruvian geographic races of *Heliconius erato* differing in mimetic colour pattern. Similar results are also evident from the coincident hybrid zone between races of its co-mimic *H. melpomene* (Mallet *et al.* 1990). Recent studies have shown that virtually no genomic differences exist across these Peruvian hybrid zones, except at fixed differences in a handful of genomic regions controlling the colour pattern and mimicry shift (Counterman *et al.* 2010; Nadeau *et al.* 2012), suggesting that in many cases, mimetic shifts may be a first stage of divergence towards speciation. However, mating of the geographic races within each species is random in the centre of both these hybrid zones, so the contribution of the evolution of geographic races like those in Peru to speciation was still unclear. *Heliconius cydno* is a species with widespread mimicry polymorphisms, such as in *H. cydno weymeri* studied here in the southern Cauca Valley (Fig. 1). In an Ecuadorean population of this species, colour pattern contributes to assortative mating among mimetic morphs (Chamberlain *et al.* 2009).

How such racial divergence might contribute to speciation was further studied along a wet-dry ecotone in Ecuador. A narrow hybrid zone between *H. (erato) himera* and *H. erato cyrba*, which were previously regarded as conspecific, was rather different in character than the Peruvian hybrid zones in *H. erato* and *H. melpomene*. In particular, there are strong differences in mtDNA and

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Fig. 1 Mimetic races of *Heliconius cydno* and its Müllerian comimics in the Cauca Valley of Colombia. Left side, top to bottom: *Heliconius cydno cydnides*, *H. cydno weymeri* form 'weymeri', *H. cydno weymeri* form 'gustavi'. Right side, co-mimics: *H. eleuchia eleusinus*, *Elzunia humboldti*, *H. (erato) chesteronii*. Photograph by Carlos Arias.

nuclear markers across this hybrid zone. Within the hybrid zone multilocus genotypes, including colour pattern loci, form a bimodal distribution. Phenotypic hybrids between the taxa, although present, form only about 10% of the population in the centre of the hybrid zone. This is largely due to assortative mating and ecological differences between the taxa, because no hybrid inviability or sterility was found (Jiggins *et al.* 1997; McMillan *et al.* 1997). Clearly, considerable progress towards speciation has been achieved because the bimodality of the genotypic distribution shows that two clusters of genotypes coexist without collapsing, albeit in a narrow zone of contact.

As a result, *H. himera* and *H. erato* were thereafter classified as separate species.

In Colombia, there is a somewhat comparable hybrid zone along another wet–dry ecotone between two taxa also formerly considered geographic races of *H. erato*: *H. (e.) chesteronii* and *H. e. venus*. Although considerable hybridization is reported in the hybrid zone, hybrids again remain rare (25%) compared to 'pure' colour pattern phenotypes, and both mtDNA and nuclear markers also differ between the taxa (Arias *et al.* 2008).

It is extremely difficult for foreigners to obtain permits to collect wild species for DNA-based work in Colombia, and there have been, at least until recently, public order difficulties across much of that country. Mauricio Linares, the senior Colombian author of the current paper, has endured a number of life-threatening situations to obtain valuable data on Colombian *Heliconius* hybrid zones. For a long time, his research team has been studying a major hybrid zone between two mimetic races, *H. cydno cydnides* and *H. c. weymeri* in the Cauca Valley, sandwiched between the Central and Western Cordilleras of the Andes (Fig. 1). The current study, from an all Latin American group, shows some extremely interesting patterns from this hybrid zone. The two races show asymmetrical assortative courtship, and there is also clear mtDNA as well as some nuclear genetic differences across the zone. Yet, hybrids in the centre of the zone appear abundant, giving no evidence for phenotypic bimodality: hence, the authors' designation of the hybrid zone as 'unimodal' (Arias *et al.* 2012). Along the continuum of speciation, this *H. cydno* hybrid zone therefore sits between bimodal hybrid zones like those of *H. himera* and *H. erato*, and classical clinal hybrid zones like those between Peruvian races of *H. erato* or *H. melpomene* (Table 1).

The authors also find that the mtDNA haplotypes of the southern race (*H. c. weymeri*) are closely related to those of the Pacific coastal race *H. cydno zelande*, whereas the northern *H. c. cydnides* groups with races further east, from the Magdalena Valley. According to the authors, this argues

Table 1 The speciation continuum in *Heliconius* butterflies: examples of hybrid zones among species and geographic races

Taxon 1	Taxon 2	Locality	Unimodal/ Bimodal	mtDNA divergence	Nuclear divergence	Taxonomic status	References
<i>H. erato favorinus</i>	<i>H. erato emma</i>	NE Peru	Unimodal	No	Colour pattern loci only	Geographic races	Mallet <i>et al.</i> (1990) and Counterman <i>et al.</i> (2010)
<i>H. melpomene amaryllis</i>	<i>H. melpomene aglaope</i>	NE Peru	Unimodal	No	Colour pattern loci only	Geographic races	Mallet <i>et al.</i> (1990) and Nadeau <i>et al.</i> (2012)
<i>H. cydno cydnides</i>	<i>H. cydno weymeri</i>	Cauca, Colombia	Unimodal	Yes	Some divergence	Geographic races	Arias <i>et al.</i> (2012)
<i>H. erato cyrbia</i>	<i>H. (erato) himera</i>	S Ecuador	Bimodal, 10% hybrids	Yes	Strong	Species	Jiggins <i>et al.</i> (1997)
<i>H. erato venus</i>	<i>H. (erato) chesteronii</i>	W Colombia	Bimodal, 25% hybrids	Yes	Yes	Species	Arias <i>et al.</i> (2008)

for the current hybrid zone forming by secondary contact as a result of the spread of *H. c. weymeri* accompanied by colour pattern divergence across passes in the Western Andes into the southern Cauca Valley. Here, it met *H. c. cydnides* that itself spread from the east. As with most phylogeographic hypotheses, this is hard to prove, especially as it has been shown that some colour patterns in *H. erato* seem to have spread behind moving contact zones while in parapatric contact with another race (Blum 2002), so potentially explaining the existence of multiple, disjunct but otherwise very similar, mimicry races up and down the Andes (Hines *et al.* 2011). Nonetheless, the existence of strong mtDNA and some nuclear divergence across this *H. cydno* contact zone argues for greater population restructuring than just a moving colour pattern cline within a continuous geographic range; the authors rightly argue that this is good evidence that the hybrid zone was achieved via secondary contact.

What is not yet quite clear is whether speciation can take place during complete overlap in sympatry. The existence of regular albeit low-level hybridisation among many sympatric *Heliconius* species (Mallet *et al.* 2007) and the high degree of sympatry among sister species (Rosser *et al.* 2012) argues that sympatric speciation may be common (Rosser 2012). Formation of the hybrid species *Heliconius heurippa* and *H. elevatus* were almost certainly also explained by sympatric transfer of colour pattern genes between species (Mavárez *et al.* 2006; *Heliconius* Genome Consortium 2012). On the other hand, the existence of a continuum of speciation in parapatric hybrid zones like those studied by Arias *et al.* (2012; Table 1) suggests that at least some of the speciation is completed in parapatric contact or perhaps sometimes even in complete geographic isolation.

Funding is getting more difficult for evolutionary studies in 'developed' countries. Meanwhile, strong economic growth in emerging industrial powers like Colombia is contributing to greatly improved strength of their own science base. Further work on problems like these will be carried out increasingly by indigenous teams like this Colombian research group, with their ready access to high biodiversity in the complex topography of the Andes and adjacent tropical rainforests. This work shows great potential to clarify remaining questions about speciation in *Heliconius* soon.

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